AMENDMENTS TO THE SPECIFICATION

IN THE SPECIFICATION

On page 18, line 23, please replace the original paragraph with the following amended paragraph:

-- RNAi (RNA interference) method is the method employing the phenomenon that double-stranded RNA administered to cells or individual inhibits the expression of the gene (synthesis of protein) having the same sequence. Since RNAi method selectively suppresses the expression of the target gene by destroying the target gene or mRNA, it attracts attention as a method effective for analysis of function of the gene. Further, since RNAi method targets the causative gene *per se* of the disease or the disease-related gene *per se*, it also attracts attention as a method close to causal therapy. Double-stranded RNAs having 21-23 base units used in RNAi are also called siRNA. siRNAs may easily be designed. For example, to avoid the binding site of transcription factor, the first AA, CA, GA or TA is found from the site downstream of the start codon in the translated region by not less than 50 bases, and the 21 to 23 bases including the subsequent 19 to 21 bases is selected. The selected nucleotide sequence preferably has a GC contents of 30 to 70%, more preferably 50%, and preferably does not contain GGG or CCC. Then it is confirmed that the selected nucleotide sequence is specific to the target gene by BLAST search or the like. Since an siRNA is also a long chain composed of four types of base units, the siRNA against any target gene may be simply and routinely synthesized. By virtue of recent progress, use of siRNA as a novel type of therapeutic method can be devised now. More particularly, as siRNAs for human casein kinase 2 β unit, for example, 5'-cuaccgacaagcucuagactt-3'(SEQ ID NO: 20) and 5'-gucuagagcuugucgguagtt-3'(SEQ ID NO: 21) may be used. Alternatively, for

example, 5'-cuaccgacaagcucuagacat--3'(SEQ ID NO: 22) and 5'-gucuagagcuugucgguagtg-3'(SEQ ID NO: 23) may be used. For rat, 5'--aucuuacuggacucaaugatt--3'(SEQ ID NO: 24) and 5'-gauggcuguucgagaucugtt-3'(SEQ ID NO: 25), for example, may be used. Alternatively, it is preferred to use an optional similar oligonucleotide or its analogue, which may be prepared by those skilled in the art based on the knowledge of siRNA that is preferred for the inhibition of expression of casein kinase 2, may preferably be used. By administering such an siRNA to cells or individual, expression of casein kinase 2 alone may be selectively inhibited nucleotide sequence-specifically, so that the kidney diseases may be cured. Especially, it is effective for the diseases characterized by increase of expression of casein kinase 2, such as nephritis. --

On page 34, line 24, please replace the original paragraph with the following amended paragraph:

-- In cases where the expression of enzyme activity of casein kinase 2 is used as the index, the enzyme activity of casein kinase 2 protein or partial polypeptide thereof when the enzyme substrate is made to contact with the casein kinase 2 protein or partial polypeptide thereof is detected, by using protein phosphorylation analysis method, intracellular localization change analysis method or enzyme activity analysis method. In these methods, as the enzyme substrate, any substance which can serve as an enzyme substrate of casein kinase 2 or the peptide, and usually, casein protein or a partial peptide of casein kinase 2 protein is used. Further, as mentioned above, casein kinase 2 may be obtained by various methods. For example, casein kinase 2 may be obtained by extraction and purification from organs of mammals or by separation and purification from cell culture. Alternatively, recombinant casein kinase 2 may be obtained from the culture of the transformants prepared by incorporating the

gene coding for casein kinase 2 into appropriate host cells by the known genetic engineering method. The thus obtained casein kinase 2 may be used for confirming the inhibitory activity of the substance which inhibits the enzyme activity of casein kinase 2, even if a part of the amino acid sequence thereof is deleted or substituted, or other amino acid sequence is inserted, as long as it has the enzyme activity. In a method for analyzing the enzyme activity, for example, a sample (10 ng to 0.1 mg protein) is incubated with a peptide Arg-Arg-Arg-Glu-Glu-Glu-Thr-Glu-Glu-Glu (SEQ ID NO: 26) or Arg-Arg-Glu-Glu-Glu-Thr-Glu-Glu-Glu (SEQ ID NO: 27) (0.2mM) as the enzyme substrate in a buffer (20mM MOPS, pH 7.2, 25mM β-glycerol phosphate, 5mM EGTA, 1mM sodium *o*-vanadate, 1 mM dithiothreitol, 15 mM $MgCl_2$, 0.1mM [γ-^{32}P] GTP or [γ -^{32}P]ATP, 0.002mCi) at 37°C for 10 minutes. After the incubation, 40% trichloroacetic acid is added to stop the reaction, and an aliquot of the reaction solution is taken on phosphocellulose paper (1 to 1.5 cm square, Whatman P81 or the like). The phosphorylation of the peptide which is the enzyme substrate may be measured by washing the phosphocellulose paper with 0.75% phosphoric acid 3 times for 5 minutes per wash, then further washing the paper with acetone for 5 minutes, and measuring the radioactivity of ^{32}P on the phosphocellulose paper by a scintillation (Econofluor-2, NEN or the like) (R. Roskoski, Methods Enzymol. Vol. 99, pp.3-6, (1983)). High radioactivity of ^{32}P measured here indicates high expression of the enzyme activity of the casein kinase 2 protein or a partial peptide thereof in the sample. The enzyme activity analysis method similar to this method may be carried out easily because a commercially available enzyme activity-measuring kit (Casein kinase 2 Kinase Assay, Upstate) may be used. --

On page 38, line 14, please replace the original paragraph with the following amended paragraph:

-- To rats (Wistar-Kyoto strain, male, body weight 190 to 210 g, Charles River Japan, Inc.), rabbit anti-glomerular basement membrane antibody was intravenously administered (0.3 ml/kg) to induce nephritis. To the rats of normal group, normal rabbit serum (0.3 ml/kg) was intravenously administered. On 7 to 14 days after the induction of nephritis, all rats were placed in metabolic cages, and urine excreted during 24 hours was collected and the amount of the urine was measured. The urine was then centrifuged at 3000 rpm for 15 minutes, and the urine protein in the supernatant was measured using TP Test Wako (Wako Pure Chemical). Further, after the collection of urine, the kidneys were isolated, and RNAs were extracted therefrom by a conventional method, followed by preparation of cDNAs from 1 μg of RNAs using a commercially available reverse transcription reaction kit (Invitrogen). Using 1 μl each of the synthesized cDNAs as templates, PCR was performed using the primers for casein kinase 2 α subunit and using a commercially available PCR kit (Takara) to amplify the cDNA. Primers were prepared based on the nucleotide sequence of the full length cDNA of rat casein kinase 2 α subunit. That is, as the sense primer, 5'-agaaagcttcggctaataga-3'(SEQ ID NO: 13) was used, and as the antisense primer, 5'-actgaagaaatccctgacat-3'(SEQ ID NO: 14) was used. The PCR was carried out by repeating 30 times the thermal cycle of 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 40 seconds, and the reaction was stopped finally at 4°C. Each of 10 μl aliquots of the amplified PCR products was electrophoresed on agarose gel, and the gel was subjected to ethidium bromide staining, followed by measuring the expression of the casein kinase 2 α subunit gene. This measurement was carried out by taking photographs of the detected casein kinase 2 so as to make image files of the expression of the casein kinase 2 gene, and degitalizating the image file using a software

NIH Image on Macintosh. When the PCR was carried out, a constantly expressing gene (a house keeping gene, G3PDH) was detected as an internal standard, and the results of detection of the expression of casein kinase 2 α subunit gene was compensated. --

On page 39, line 26, please replace the original paragraph with the following amended paragraph:

-- First, to rats (Wistar-Kyoto strain, male, body weight 190 to 210 g, Charles River Japan, Inc.), rabbit anti-glomerular basement membrane antibody was intravenously administered (0.3 ml/kg) to induce nephritis. To the rats of normal group, normal rabbit serum (0.3 ml/kg) was intravenously administered. After the induction of nephritis, all rats were placed in metabolic cages, and urine excreted during 24 hours was collected, and then the kidneys were isolated. RNAs were extracted from each kidney to be subjected to diagnosis and from each kidney of non-diseased state as a control by a conventional method, and cDNAs were synthesized from 1 μg of the respective RNAs using a commercially available reverse transcription reaction kit (Invitrogen). Using 1 μl each of the synthesized cDNAs as templates, PCR was performed using the primers for casein kinase 2 α subunit and using a commercially available PCR kit (Takara) to amplify the cDNA. Primers were prepared based on the nucleotide sequence of the full length cDNA of rat casein kinase 2 α subunit. That is, as the sense primer, 5'-agaaagcttcggctaataga-3'(SEQ ID NO: 13) was prepared and used, and as the antisense primer, 5'-actgaagaaatccctgacat-3'(SEQ ID NO: 14) was prepared and used. The PCR was carried out by repeating 30 times the thermal cycle of 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 40 seconds, and the reaction was stopped finally at 4°C. Each of 10 μl aliquots of the amplified PCR products was electrophoresed on agarose gel, and the gel was subjected to ethidium

bromide staining, followed by measuring the expression of the casein kinase 2 gene. This measurement was carried out by taking photographs of the detected casein kinase 2 so as to make image files of the expression of the casein kinase 2 gene, and degitalizating the image file using a software NIH Image on Macintosh. When the PCR was carried out, a constantly expressing gene (a house keeping gene, G3PDH) was detected as an internal standard, and the results of detection of the expression of casein kinase 2 α subunit gene was compensated. --

On page 42, line 7, please replace the original paragraph with the following amended paragraph:

-- First, to rats, rabbit anti-glomerular basement membrane antibody was intravenously administered (0.3 ml/kg) to induce nephritis. To the rats of normal group, normal rabbit serum (0.3 ml/kg) was intravenously administered. After the induction of nephritis, all rats were placed in metabolic cages, and urine excreted during 24 hours was collected, and then the kidneys were isolated. RNAs were extracted from each kidney to be subjected to diagnosis and from each kidney of non-diseased state as a control by a conventional method, and cDNAs were synthesized from 1 μg of the respective RNAs using a commercially available reverse transcription reaction kit (Invitrogen). Using 1 μl each of the synthesized cDNAs as templates, PCR was performed using the primers for casein kinase 2 β subunit and using a commercially available PCR kit (Takara) to amplify the cDNA. Primers were prepared based on the nucleotide sequence of the full length cDNA of rat casein kinase 2 β subunit. That is, as the sense primer, 5'-ccgcggacataaagatgagt-3'(SEQ ID NO: 11) was prepared and used, and as the antisense primer, 5'-aaaccagtgccgaagtatgc-3'(SEQ ID NO: 12) was prepared and used. The PCR was carried out by repeating 30 times the thermal cycle of 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 40 seconds, and the reaction was stopped finally at 4°C. Each of 10 μl aliquots of the

amplified PCR products was electrophoresed on agarose gel, and the gel was subjected to ethidium bromide staining, followed by measuring the expression of the casein kinase 2 gene. This measurement was carried out by taking photographs of the detected casein kinase 2 so as to make image files of the expression of the casein kinase 2 gene, and degitalizating the image file using a software NIH Image on Macintosh. When the PCR was carried out, a constantly expressing gene (a house keeping gene, G3PDH) was detected as an internal standard, and the results of detection of the expression of casein kinase 2 β subunit gene was compensated. --

On page 44, line 9, please replace the original paragraph with the following amended paragraph:

-- Using the expression of casein kinase 2 as an index, kidney disease was diagnosed. That is, using diabetic rats (Zucker strain) which spontaneously suffered from diabetic nephropathy, expression of casein kinase 2 β subunit gene in the kidney was detected by RT-PCR method, and the kidney disease was diagnosed using it as an index. Blood was sampled from the rats which spontaneously suffered from diabetic nephropathy (Zucker fa/fa strain, 6 months old, Charles River Japan, Inc.) and from the control rats which did not spontaneously suffered from diabetic nephropathy (Zucker Lean strain, 6 months old, Charles River Japan, Inc.), and then kidneys were isolated from the rats. From each kidney to be subjected to diagnosis and from each control kidney which was not diseased, RNAs were extracted by a conventional method, and cDNAs were synthesized from 1 μg of the respective RNAs using a commercially available reverse transcription reaction kit (Invitrogen). Using 1 μl each of the synthesized cDNAs as templates, PCR was performed using the primers for casein kinase 2 β subunit and using a commercially available PCR kit (Takara) to amplify the cDNA. Primers were prepared based on the nucleotide sequence of the full length cDNA of rat casein kinase 2 β subunit.

That is, as the sense primer, 5'-ccgcggacataaagatgagt-3'(SEQ ID NO: 11) was prepared and used, and as the antisense primer, 5'-aaaccagtgccgaagtatgc-3'(SEQ ID NO: 12) was prepared and used. The PCR was carried out by repeating 30 times the thermal cycle of 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 40 seconds, and the reaction was stopped finally at 4°C. Each of 10 μl aliquots of the amplified PCR products was electrophoresed on agarose gel, and the gel was subjected to ethidium bromide staining, followed by measuring the expression of the casein kinase 2 gene. This measurement was carried out by taking photographs of the detected casein kinase 2 so as to make image files of the expression of the casein kinase 2 gene, and degitalizating the image file using a software NIH Image on Macintosh. When the PCR was carried out, a constantly expressing gene (a house keeping gene, G3PDH) was detected as an internal standard, and the results of detection of the expression of casein kinase 2 β subunit gene was compensated. --

On page 49, line 16, please replace the original paragraph with the following amended paragraph:

-- The relationship between casein kinase 2 protein content and enzyme activity was analyzed. That is, each of the casein kinase 2 (Upstate) having varying protein contents (0, 10 and 20 ng protein, respectively) was incubated in 0.05 ml of a buffer (20mM MOPS, pH7.2, 25mM β-glycerose phosphate, 5mM EGTA, 1mM sodium *o*-vanadate, 1mM dithiothreitol, 15mM $MgCl_2$, 0.1mM [γ-^{32}P]ATP, 0.002 mCi, Amersham) containing Arg-Arg-Arg-Glu-Glu-Glu-Thr-Glu-Glu-Glu (SEQ ID NO: 26) (10nmol, 0.2mM, Upstate) which is an enzyme substrate, at 37°C for 10 minutes. After the incubation, 0.025 ml of 40% trichloroacetic acid was added to stop the reaction, and 0.025 ml aliquot of the reaction solution was taken on phosphocellulose paper (1 cm x 1 cm). The phosphocellulose paper

was washed three times with 25 ml of 0.75% phosphoric acid for 5 minutes per wash, and then washed with 25 ml of acetone for 5 minutes, followed by measurement of the radioactivity of ^{32}P on the phosphocellulose paper by scintillation. --

On page 50, line 20, please replace the original paragraph with the following amended paragraph:

-- This example was carried out for studying the effect of administration of an antisense oligonucleotide against casein kinase 2 α subunit, so as to confirm the utility of the present invention. To rats (Wistar-Kyoto strain, male, body weight 190 to 210 g), rabbit anti-glomerular basement membrane antibody was intravenously administered (0.3 ml/kg) to induce nephritis. To the kidney tissues of the nephritis rats, an antisense oligonucleotide 5'-GTAATCATCTTGATTACCCCA-3' (SEQ ID NO: 1) which selectively inhibits expression of casein kinase 2 α subunit, or a sense oligonucleotide 5'-TGGGGTATCAATCAAGATGATTAC-3' (SEQ ID NO: 28) which does not inhibit the expression of casein kinase 2 α subunit was administered at a dose of 12 μg/day which is said to be appropriate for the inhibition of casein kinase 2 using Alzet pumps (0.25 μl/hour, 1002, Alzet) connected through polyethylene tubes(PE-10), continuously from one day before the induction of nephritis to the 7th day. The nucleic acid molecules of the oligonucleotides were S-oligonucleotides containing phosphorothioate moieties, and a cationic liquid (Polyplus transfection) was used for making the uptake of the oligonucleotide easier. More particularly, the groups used in the experiment were normal group (normal rats to which the antisense oligonucleotide against casein kinase 2 α subunit and the antisense oligonucleotide were not administered, n=8), nephritis control group (nephritis rats to which the antisense oligonucleotide against casein kinase 2 α subunit and the antisense oligonucleotide were not

administered, n=8), nephritis + antisense oligonucleotide against casein kinase 2 α subunit-administered group (group of the present invention, nephritis rats to which the antisense oligonucleotide against casein kinase 2 α subunit was administered, n=4), and nephritis + sense oligonucleotide of casein kinase 2 α subunit-administered group (negative control group, nephritis rats to which the sense oligonucleotide of casein kinase 2 α subunit was administered), totally four groups. On the 7th day from the induction, all rats were placed in metabolic cages, and urine excreted during 24 hours was collected and the amount of the urine was measured. The urine was then centrifuged at 3000 rpm for 15 minutes, and the urine protein in the supernatant was measured. Further, after the collection of urine, the kidneys were isolated, proteins were extracted from each kidney tissue by a conventional method, and the proteins (1 mg/ml, 20 μl) were electrophoresed (PAGERUN, ATTO, 40mA, 84min) on 12.5% polyacrylamide gel (PAGEL, ATTO). The electrophoresed proteins were blotted (100V, 90 min) on a PVDF membrane (Millipore). The membrane was blocked (Blocking Ace, Dainippon Pharmaceutical, 4°C, 1 hour), and washed three times (10 min) with PBS containing Tween 20. Then the membrane was incubated (at room temperature for 1 hour) with an anti-casein kinase 2 α subunit (originated from goat, Santa cruz, 100-fold diluted) as the primary antibody. After the incubation and after washing (10 min) the membrane 3 times with PBS containing Tween 20, the membrane was incubated (at room temperature for 1 hour) with an anti-goat IgG antibody labeled with HRP (Santa cruz, 1000-fold diluted used as the secondary antibody). After the incubation, the amounts of the casein kinase 2 α subunit protein were measured, respectively, using ECL reagent (Amersham). This measurement was carried out by taking photographs of the detected casein kinase 2 so as to make image files of the amount of the casein kinase 2 protein, and degitalizating the image file using a software NIH Image on Macintosh. --

On page 53, line 11, please replace the original paragraph with the following amended paragraph:

-- The inhibitory action of a compound, apigenin, against the enzyme activity of casein kinase 2 was studied. Casein kinase 2 (protein amount: 10 ng, Upstate) was incubated in 0.05 ml of a buffer (20mM MOPS, pH7.2, 25mM β-glycerose phosphate, 5mM EGTA, 1mM sodium *o*-vanadate, 1mM dithiothreitol, 15mM $MgCl_2$, 0.1mM [γ-^{32}P]ATP, 0.002 mCi, Amersham) containing Arg-Arg-Arg-Glu-Glu-Glu-Thr-Glu-Glu-Glu (SEQ ID NO: 26) (10nmol, 0.2mM, Upstate) which is an enzyme substrate and containing apigenin at a concentration within the range of 0.1 μM to 100 μM, at 37°C for 10 minutes. After the incubation, 0.025 ml of 40% trichloroacetic acid was added to stop the reaction, and 0.025 ml aliquot of the reaction solution was taken on phosphocellulose paper (1 cm x 1 cm). The phosphocellulose paper was washed three times with 25 ml of 0.75% phosphoric acid for 5 minutes per wash, and then washed with 25 ml of acetone for 5 minutes, followed by measurement of the radioactivity of ^{32}P on the phosphocellulose paper by scintillation. The ratio of the radioactivity of ^{32}P measured for each buffer containing the inhibitor at the respective concentration to the radioactivity of ^{32}P measured for the buffer not containing apigenin was expressed in terms of % taking the radioactivity measured for the buffer not containing apigenin as 100%, and the effect against the functional activity or against the effect of casein kinase 2 was determined. --